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Current concepts on oxidative/carbonyl stress, inflammation and epigenetics in pathogenesis of chronic obstructive pulmonary disease

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Abstract

Chronic obstructive pulmonary disease (COPD) is a global health problem, and current therapy for COPD is poorly effective and the mainstays of pharmacotherapy are bronchodilators. A better understanding of the pathobiology of COPD is critical for the development of novel therapies. In the present review, we have discussed the roles of oxidative/aldehyde stress, inflammation/immunity, and chromatin remodeling in the pathogenesis of COPD. Imbalance of oxidant/antioxidant balance caused by cigarette smoke and other pollutants/biomass fuels plays an important role in the pathogenesis of COPD by regulating redox-sensitive transcription factors (e.g. NF- κ B), autophagy and unfolded protein response leading to chronic lung inflammatory response. Cigarette smoke also activates canonical/alternative NF- κ B pathways and their upstream kinases leading to sustained inflammatory response in lungs. Recently, epigenetic regulation has been shown to be critical for the development of COPD because the expression/activity of enzymes that regulate these epigenetic modifications have been reported to be abnormal in airways of COPD patients. Hence, the significant advances made in understanding the pathophysiology of COPD as described herein will identify novel therapeutic targets for intervening COPD.

Keywords

COPD; oxidants; smokers; inflammation; epigenetics; NF- κ B; SIRT1

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major and increasing global health problem and is the fourth commonest cause of death in the developed countries. It is a disabling condition associated with progressive breathlessness. COPD will account for over 6 million deaths per year by 2020 and is predicted to take a leap from the sixth- to the third-leading cause of death worldwide. In America, COPD affects 9% of residents aged 60 years

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and above and is ranked fourth in the recent morbidity survey of the elderly population. It is estimated that approximately 23.4 million people in the USA have COPD and the health burden is \$36.1 billions per year. The burden of COPD for the patient is high as patients experience a poorer quality of life, suffer from comorbidities (3.7 comorbidities per patient), and direct healthcare amounted to 20.9 billion dollar in the USA in 2004.

Cigarette smoke is the major risk factor for the development of COPD. It is likely to account for ~80%–90% of COPD cases in USA (Sethi and Rochester, 2000). Cigarette smoke contains an estimated 10^{15} – 10^{17} oxidants/free radicals and ~4,700 different chemical compounds, including reactive aldehydes and quinones, per puff (Church and Pryor, 1985). Cigarette smoke is the primary cause of COPD (emphysema and chronic bronchitis) characterized by accelerated decline in lung function, inflammation and premature aging of the lung. However, only 10%–20% of the smokers develop COPD pointing at an additional risk factor such as genetic susceptibility, e.g. the polymorphisms in genes coding for (anti-) proteases like alpha-1 antitrypsin (1AAT), a disintegrin and metalloproteinase 33 (ADAM33), or antioxidant superoxide dismutase (SOD), and pro-inflammatory mediators tumor necrosis factor- α (TNF- α) (Harrison *et al.*, 1997; Keatings *et al.*, 2000; Sandford *et al.*, 2001; Kucukaycan *et al.*, 2002; Celedon *et al.*, 2004; Young *et al.*, 2006). Other noxious environmental gases/particles such as NO₂, SO₂, and particulate matters, as well as exposure to second hand tobacco hand smoke and biomass fuel can also cause oxidative stress and trigger inflammatory responses in the lungs in a susceptible population. Cessation of smoking reduces progression of the disease only if applied early and has little effect after significant symptoms ensues. At present, no effective treatment exists to halt the decline in lung function in smokers who get the disease. This in turn reflects a lack of understanding of the specific cellular and biochemical pathways triggered in the lung by tobacco smoke. Thus, it is essential that COPD research should focus on improving our understanding of the specific cellular and biochemical injury induced by tobacco smoke within the lung. Most treatments for COPD are mainly palliative, and no single therapy exists that can halt the decline in lung function or progressive destruction of the airways. The mainstays of pharmacotherapy are bronchodilators (to relieve the symptoms of bronchoconstriction), corticosteroids (to reduce the airway inflammation), and combination of bronchodilators with corticosteroids. However, current corticosteroid therapy in COPD is poorly effective (Barnes *et al.*, 2004). This has prompted an intense search for new anti-inflammatory therapeutic targets based on a better understanding of the underlying pathophysiology of COPD.

PATHOGENESIS

COPD is a preventable and treatable disease with some significant extrapulmonary effects that may contribute to severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible, and the airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases (Rabe *et al.*, 2007). COPD can be classified into 4 classes of severity based on lung function [GOLD Guidelines]. Emphysema, chronic bronchitis with airway obstruction, and small airways disease are the distinct phenotypes of COPD, but most patients show a combination of different phenotypes. Emphysema is characterized by a Th1 type inflammation, destruction of the alveolar septa, loss of elastic recoil, airspace enlargement and hence loss of gas diffusion capacity (Wright and Churg, 2006). Chronic bronchitis affects the large airways by airway inflammation, goblet cell hyperplasia and mucus hypersecretion. In addition to decreased lung function, these patients experience chronic sputum production, coughing and often dyspnoea. Small airways disease mainly affects the bronchioles featuring airway inflammation and metaplasia of Clara cells.

The pathogenesis of COPD involves several pathogenetic processes, such as oxidative stress, inflammation, protease/antiprotease imbalance, alteration in immunity (autoantibody production), apoptosis, alteration of cell proliferation, and cellular senescence/aging, induced by air pollutants, modified by genetic factors, and exacerbated by virus and bacteria (Shapiro and Ingenito, 2005; El Moussaoui *et al.*, 2008; Kang *et al.*, 2008; Tudor and Yun, 2008; MacNee and Tudor, 2009; Sethi *et al.*, 2009). This review focuses on specific molecules that regulate oxidative stress, inflammatory response, and epigenetics and their mechanisms/consequences so as to provide the possible therapies against these targets for intervention in COPD.

OXIDATIVE AND ALDEHYDE/CARBONYL STRESS IN COPD

Formation of reactive and unstable free radicals such as superoxide anion ($O_2^{\bullet-}$), nitric oxide, peroxynitrite ($ONOO^-$) and hydroxyl radicals ($\bullet OH$) lead to a series of chain reactions which yields uncontrolled (if not ablated) tissue destruction as a result of oxidation. The importance of oxidative stress has been confirmed by several studies that have identified the presence of free radical biomarkers in patients with COPD. Increased level of 8-hydroxy-deoxyguanosine was detected in urine of COPD patients, and elevated level of 3-nitrotyrosine and $F_{2\alpha}$ isoprostanes occurred in lungs of COPD patients, and these markers demonstrated a strong correlation with disease severity as measured by forced expiratory volume in the first second (FEV_1) (Ichinose *et al.*, 2000; Rahman *et al.*, 2002; Igishi *et al.*, 2003). Furthermore, the levels of lipid peroxidation products 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA) were increased in lungs of patients with COPD, and this increase was negatively correlated with lung function (Rahman *et al.*, 2002; Harju *et al.*, 2004; Ryttila *et al.*, 2006). These reactive aldehydes deplete thiol pool, carbonylate proteins or form aldehyde-protein adducts leading to alteration of protein function and causing a variety of cellular and biochemical effects including immunogenicity and proteolysis thereby inducing lung inflammatory and autoimmune responses, and injury (Fig. 1). Thus, redressing of oxidant/antioxidant imbalance and reducing lipid peroxidation will be able to prevent the progression of COPD as shown previously (Kinnula, 2005; De Boer *et al.*, 2007b).

Exogenous and endogenous ROS production

There are essentially two sources of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that the lungs are exposed to, environmental and cellular. The single most important environmentally derived ROS in driving the pathogenesis of COPD is cigarette smoke. ROS/RNS are also generated by several inflammatory and structural cells of the airways. One of the features of COPD is an inflammatory-immune response, characterized by activation of epithelial cells, and resident macrophages, and the recruitment and activation of neutrophils, monocytes, and B- and T- lymphocytes. Inflammatory cells once recruited in the airspace become activated and generate ROS in response to a sufficient level of a secretagogue stimulus (threshold condition). The principle ROS-generating enzyme in inflammatory cells is NADPH oxidase. Other enzyme systems such as the xanthine/xanthine oxidase system and the heme peroxidases are also involved in COPD (Rahman *et al.*, 1996; Pinamonti *et al.*, 1998). Similarly, RNS in the form of nitric oxide (NO) production is generated by nitric oxide synthase. Moreover, nitric oxide will form the more potent and damaging peroxynitrite molecules in the presence of superoxide anion. Interestingly, we and others have shown that disruption of $p47^{phox}$ and $gp91^{phox}$, the components of NADPH oxidase, resulted in airspace enlargement in mice (Kassim *et al.*, 2005; Yao *et al.*, 2008b) suggesting ROS-derived from NADPH oxidase participate in signaling pathway of tissue homeostasis, and the use of NADPH oxidase inhibitor for redressing the oxidant/antioxidant imbalance in COPD may be deleterious.

Antioxidant defense in COPD

Under normal condition, the lungs have well coordinated and efficient endogenous antioxidant defense systems, which protect against the injurious effects of oxidants by electron transfer, enzymatic removal, scavenging and by keeping transition metal ions tightly sequestered. Furthermore, a variety of enzymes including aldehyde dehydrogenase and aldo-keto reductase, which are responsible for the detoxification of reactive aldehydes such as acetaldehyde and acrolein, were significantly induced in mouse lung exposed to cigarette smoke (Rangasamy *et al.*, 2004), but their role in lungs of chronic smokers and in pathogenesis of COPD is not known. These phase I enzymes decarbonylate proteins and thereby reverse their post-translational modifications caused by reactive aldehydes (4-HNE, acrolein, and acetaldehyde). Nevertheless, it has been shown that increased ROS production and reduced endogenous antioxidant defense has been reported in several lung diseases including COPD (Repine *et al.*, 1997; Macnee and Rahman, 1999; Rahman and MacNee, 2000; Tomaki *et al.*, 2007). In patients with COPD, this balance may be disturbed due to mutations in genes encoding for antioxidant enzymes, such as extracellular SOD, glutathione S-transferase M1 (GSTM1), GSTT1, GSTP1, and glutamate cysteine ligase (GCL) (Harju *et al.*, 2002; He *et al.*, 2002; Kinnula, 2005; Juul *et al.*, 2006; Young *et al.*, 2006; Mak *et al.*, 2007; Siedlinski *et al.*, 2008). Recent animal studies showed that Cu/Zn SOD protected cigarette smoke-, elastase- and ceramide-induced emphysema in mice (Foronjy *et al.*, 2006; Petrache *et al.*, 2008). Likewise, overexpression of extracellular SOD attenuated acute cigarette smoke-mediated lung inflammatory response and elastase-induced emphysema in mice (Yao *et al.*, unpublished work). Indeed, the dead space volume/total lung capacity was increased which reflects a reduction of ventilation efficiency in extracellular SOD deficient mice compared to WT mice (Ganguly *et al.*, 2007). Furthermore, acute reduction of extracellular SOD using cre-lox homologous recombination (conditional knockout) led to an increase in lung superoxide, marked inflammatory cell infiltration, the arterial-alveolar gradient, respiratory acidosis, histological changes similar to those observed in adult respiratory distress syndrome, and 85% mortality (Gongora *et al.*, 2008). Treatment with the SOD mimetic MnTBAP and intranasal administration of SOD-containing polyketal microparticles reduced mortality, prevented the histological alterations, and reduced lung superoxide levels (Gongora *et al.*, 2008). In addition, the polymorphisms of extracellular SOD gene was associated with reduced lung function or lower risk of COPD (Young *et al.*, 2006; Dahl *et al.*, 2008). These results suggested that antioxidant therapy would seem to be a logical therapeutic approach in COPD. The use of a variety of antioxidants, such as SOD mimetics, mucolytic agents, such as N-acetyl-L-cysteine (NAC, a cellular precursor of GSH) and erdosteine, has met with varying success in patients with COPD (Dekhuijzen, 2004; Moretti *et al.*, 2004; Decramer *et al.*, 2005; van Overveld *et al.*, 2005).

It has been shown that the protein but not mRNA level of nuclear erythroid-related factor 2 (Nrf2), a transcription factor upregulating phase II genes, was decreased in lungs of patients with COPD/emphysema, and deficiency of Nrf2 resulted in enhanced susceptibility to cigarette smoke- and elastase-induced emphysema in mice which is associated with more pronounced oxidative stress in lungs (Rangasamy *et al.*, 2004; Iizuka *et al.*, 2005; Ishii *et al.*, 2005; Goven *et al.*, 2008; Malhotra *et al.*, 2008; Suzuki *et al.*, 2008; Cho and Kleeberger, 2009; Singh *et al.*, 2009). Therefore, modulation of Nrf2 would be expected to have significant beneficial effects in cigarette smoke-mediated oxidative stress and lung inflammation. The reduction of Nrf2 was corroborated with our findings showing attenuation of posttranslational modification of Nrf2 by resveratrol, a polyphenolic phytoalexin present in red wine, protected against cigarette smoke-mediated oxidative stress in human epithelial cells (Kode *et al.*, 2008). It was associated with nuclear translocation of Nrf2, thereby leading to induction of GCL, a rate-limiting enzyme for the synthesis of glutathione. Silencing of DJ-1, a stabilizer of Nrf2, in mouse lungs, mouse embryonic

fibroblasts and Beas2B cells impaired antioxidant induction in response to cigarette smoke (Malhotra *et al.*, 2008). Genetic deletion of KEAP1, a cytosolic inhibitor of Nrf2, in Clara cells attenuated cigarette smoke-induced inflammation and oxidative stress in mouse lung (Blake *et al.*, 2009). Therefore, enhancement of DJ-1 would stabilize Nrf2 protein promoting the expression of key antioxidant enzymes in response to cigarette smoke. It is interesting to note that the level of DJ-1 was reduced and post-translationally modified in COPD lungs but not in those of normal smokers. Recently, it has been shown that wild-type mice exposed to cigarette smoke, when treated with CDDO-Im [imidazole and methyl ester derivative of 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO)] exhibited significant reductions in both oxidative stress and alveolar destruction with increased transcriptional induction of multiple Nrf2-regulated antioxidant genes (Biswal *et al.*, 2008; Sussan *et al.*, 2009). However, Nrf2 knockout mice had no significant reduction in alveolar destruction following treatment with CDDO-Im (Biswal *et al.*, 2008; Sussan *et al.*, 2009). These data suggest that activation of the Nrf2 pathway, stabilization of DJ1 and reversing posttranslational carbonyl modifications of Nrf2, Keap1 and DJ1 using different approaches can be developed to protect lungs against cigarette smoke/oxidative stress-induced inflammatory response and emphysema.

Oxidative/aldehyde stress-mediated neurogenic inflammation in lungs

Transient receptor potential cation channel, subfamily A, member 1 (TRPA1) is an excitatory ion channel expressed by a subpopulation of primary afferent somatosensory neurons that contain substance P and calcitonin gene-related peptide. TRPA1 can be activated by a range of highly reactive chemicals, such as H₂O₂ and α,β -unsaturated aldehydes [acrolein, crotonaldehyde, and 4-hydroxy-2-nonenal (4-HNE)] eliciting pain or promoting immediate protective responses (Trevisani *et al.*, 2007; Andersson *et al.*, 2008). These reactive chemicals, contained in cigarette smoke or generated by lipid peroxidation in lungs of patients with COPD, are responsible for initiating and maintaining lung inflammatory response (Rahman *et al.*, 2002; Facchinetti *et al.*, 2007; Borchers *et al.*, 2008). Recent studies showed that disruption of TRPA1 gene attenuated H₂O₂- and hypochlorite-induced respiratory depression as well as oxidant-induced pain behavior suggesting TRPA1 is a major neuronal sensor for H₂O₂ and hypochlorite in the airways (Bessac *et al.*, 2008). Most importantly, cigarette smoke has the unique ability to excite capsaicin-sensitive primary sensory neurons by activating TRPA1 to induce neurogenic inflammation in the airway, and acrolein and crotonaldehyde are major mediators in cigarette smoke-induced activation of TRPA1-expressing neurons (Andre *et al.*, 2008). Therefore, topical application of specific TRPA1 antagonists to airways would benefit those peoples who are exposed to pollutants, especially with manifest airways hypersensitivities (Simon and Liedtke, 2008).

Oxidative stress-mediated lung cell autophagy and apoptosis in COPD

Autophagy is a dynamic process responsible for the turnover of cellular organelles and long-living proteins, which has been suggested to be an essential function to maintain cell homeostasis and confer adaption to adverse environment. It has been shown that accumulation of ROS led to autophagy to overcome oxidative stress (Scherz-Shouval *et al.*, 2007a; Scherz-Shouval *et al.*, 2007b). This pathway is involved in the removal and degradation of damaged mitochondria and oxidized proteins. For example, sirtuin 1 (SIRT1), a class III histone deacetylase, is suggested to clear the old and damaged mitochondria by inducing autophagy. SIRT1 also increases the supply of new mitochondria by activating peroxisome proliferator-activated receptor γ coactivator-1 α (PGC- α), the mitochondrial biogenesis regulator (Lee *et al.*, 2008). However, excessive autophagy will lead to cell death. A recent study showed that increased autophagy occurred in lungs of patients with COPD, in mouse lung tissues exposed to cigarette smoke, and in cells treated with cigarette smoke extract (Chen *et al.*, 2008; Ryter *et al.*, 2009) which may be due to the

reduction of SIRT1 in COPD (Rajendrasozhan *et al.*, 2008b; Nakamaru *et al.*, 2009) suggesting a pivotal role of autophagy in the pathogenesis of COPD. Overexpression of extracellular SOD attenuated the levels of early growth response protein-1 (Egr-1) gene and protein which is an important transcription factor in autophagy in lungs in response to hypoxia (Nozik-Grayck *et al.*, 2008). It is interesting to note that inhibition of histone deacetylase (HDAC, in particular HDAC6) activity resulted in the complex formation of Egr-1 with E2F-4, leading to increased expression of microtubule-associated protein light chain 3 (LC3), a best characterized autophagy protein (Chen *et al.*, 2008). Therefore, cigarette smoke-mediated decrease of HDAC activity leads to the transcriptional activation of Egr-1 and E2F-4, thereby inducing autophagic cell death. These results suggested that modulation of autophagic pathway may be beneficial in the intervention of COPD.

Oxidative stress-induced unfolded protein response in COPD

The unfolded protein response (UPR) is activated in response to an accumulation of unfolded or misfolded proteins in the lumen of endoplasmic reticulum (ER). The UPR has two primary aims: initially to restore normal function of the cell by preventing protein translation and activate the signaling pathways that increase the production of molecular chaperones involved in protein folding. When these objectives are not achieved within a certain time lap or the disruption is prolonged, the UPR is to initiate apoptosis. During ER stress, a chaperone Bip preferentially binds to unfolded or misfolded protein, leading to disassociation of Bip from inositol-requiring 1 α (IRE α), double-stranded RNA-dependent protein kinase-like ER kinase (PERK) and activating transcription factor 6 (ATF6) which are the initiators of the three main signaling cascades of UPR. The activation of these three pathways leads to transcription of UPR target genes encoding factors involved in amino-acid biosynthesis, the antioxidative stress response, apoptosis and inflammation (Gargalovic *et al.*, 2006; Zhang and Kaufman, 2008).

Recently, the expression of UPR markers such as ER chaperones, GRP78, calreticulin, ER foldase and protein disulfide isomerase (PDI) were shown to be upregulated in lungs of chronic cigarette smokers (Kelsen *et al.*, 2008). This was confirmed by *in vitro* studies showing the UPR was activated by gas phase and aqueous extract of cigarette smoke in normal and malignant human lungs cells and mouse fibroblast cells (Hengstermann and Muller, 2008; Jorgensen *et al.*, 2008). Moreover, several downstream targets of the UPR including antioxidant gene, thioredoxin-dependent peroxidase reductase were also increased in lungs of these smokers. These results suggest that cigarette smoke-mediated UPR may be a protective mechanism defending the lung against deleterious effects of cigarette smoke by upregulating antioxidant molecules or down-regulating pro-inflammatory proteins (Jorgensen *et al.*, 2008; Kelsen *et al.*, 2008). Indeed, Nrf2 was increased along with an increase of GRP78, ATF4 and phosphorylated eIF2 α in human bronchial epithelial cells (16-HBE) in response to cigarette smoke extract (Kelsen *et al.*, 2008). Furthermore, Nrf2 was a direct substrate of PERK, and PERK-dependent Nrf2 phosphorylation triggers a dissociation of Nrf2/Keap1 complexes and subsequent Nrf2 nuclear translocation (Cullinan *et al.*, 2003; Cullinan and Diehl, 2004). It also has been shown that activation of UPR may decrease the inflammatory response to cigarette smoke since S100-A9/calgranulin C, an inflammatory protein, was down-regulated in lungs of chronic smokers (Kelsen *et al.*, 2008). This was corroborated by the findings that inhibition of UPR in IRE1 β knockout mice augmented colonic inflammation in response to application of irritant chemicals to the colonic mucosa (Bertolotti *et al.*, 2001). However, the nature of stimuli that initiated the UPR in smokers is not known. It has been shown that a variety of oxidant stimuli induced a UPR by increasing cytosolic calcium and interfering with protein folding directly (Liu *et al.*, 1998; van der Vlies *et al.*, 2002; Harding *et al.*, 2003; Hung *et al.*, 2003). Importantly, NAC and reduced glutathione (GSH), the free radical scavengers, attenuated cigarette smoke-induced

phosphorylation of eIF2 α in A549 cells (Jorgensen *et al.*, 2008). Therefore, ROS/aldehydes present in cigarette smoke may contribute to the UPR in lungs of smokers, and antioxidants and thiol compounds will ameliorate UPR leading to an attenuation of cell injury in response to cigarette smoke.

INFLAMMATORY RESPONSE IN COPD

The chronic inflammation of COPD is characterized by an accumulation of neutrophils, macrophages, B cells, lymphoid aggregates, CD4⁺, CD8⁺ T cells, and eosinophils, particularly in the small airways (Turato *et al.*, 2002; Hogg, 2004a; Saha and Brightling, 2006; Siva *et al.*, 2007) (Fig. 2) and the degree of inflammation increases with the severity of disease as classified by the GOLD guidelines (Hogg *et al.*, 2004b).

Innate and adaptive immunity: new concept in pathogenesis of COPD

The innate defense system in lungs provides a rapid and initial response that can be triggered by varieties of stimuli, but lacks in specificity, and has no memory. The cell components of the innate immune system include neutrophils, macrophages, natural killer (NK) cells, basophils, mast cells, eosinophils and others (Fig. 2). Cigarette smoke interferes with the innate defense system by increasing mucus production, reducing mucociliary clearance, disrupting the epithelial barrier and recruiting monocytes/macrophages and neutrophils into the damaged lung tissue. These cells play an important role in the pathogenesis of COPD through their potential release of ROS, cytokines, chemokines, elastase, and metalloproteinase in response to cigarette smoke. Recent study showed that the aberrant and persistent NK cell group 2D (NKG2D) ligand expression in the pulmonary epithelium contributes to the development of COPD by activating its receptor on NK cells and CD8⁺ cells (Borchers *et al.*, 2009). This suggests that the communication of pulmonary epithelium with the immune system plays an important role in maintaining the integrity of lung tissue and its aberration leads to altered inflammatory response. Cigarette smoke also stimulates the cellular and humoral components of the adaptive immune response to provide a much more specific reaction, which has precise memory for foreign materials previously exposed to lungs (Fig. 2). However, it remains unclear why cells of adaptive immunity including B lymphocytes, CD4⁺ and CD8⁺ T lymphocytes accumulate in lungs of smokers with COPD. Organization of recruited lymphocytes into lymphoid follicles and the presence of oligoclonal lymphocytes indicate that lymphocyte recruitment is a result of a targeted, antigen-specific adaptive immune response, rather than of nonspecific trafficking of lymphocytes to the lung (Hogg *et al.*, 2004b; Sullivan *et al.*, 2005; van der Strate *et al.*, 2006; Borchers *et al.*, 2007; Borchers *et al.*, 2008; Motz *et al.*, 2008). Dendritic cells, antigen-presenting cells, play an important role in the initiation and maintenance of adaptive immune responses. Recent study showed that cigarette smoke impairs the normal maturation of dendritic cells, and recruits a large number of circulating immature dendritic cell precursors to the bronchial mucosa and lung parenchyma (Tsoumakidou *et al.*, 2008). These non- or partially matured and/or functionally impaired pulmonary dendritic cells reach the draining lymph nodes or lymphoid follicles, and cooperate with naïve lymphocytes to induce one of three predominant responses: Th1 (an increase of Th1 cytokines, and release of perforin and granzymes, associated with apoptosis and necrosis of lung cells), Th2 (an increase of IL-4 in and around mucus-secreting glands in patients with chronic bronchitis) or Treg (an increase of TGF- β and the development of tolerance to infection in lung of patients with COPD). Furthermore, dendritic cells retained antigen for presentation to T cells over long periods where such chronic inflammation eventually results in emphysema. Importantly, lung tissue damage associated with repeated cigarette smoke exposure may unmask intracellular self-protein or alter normally nonantigenic proteins to be recognized as nonself. Cross-presentation of self-antigen, such as elastin, endothelial antigens or epithelial antigens by dendritic cells to CD8⁺ T cells could lead to the development of autoimmunity

(Grumelli *et al.*, 2004; Lee *et al.*, 2007). It has been shown that carbonyl modifying proteins have effectively altered the self protein so that it becomes naïve and highly immunogenic to the immune system and hence generate antibodies against this altered non-self protein (e.g. aldehyde-cigarette smoke containing reactive aldehyde modified proteins) (Allison and Fearon, 2000) (Fig. 1). In doing this, because an antibody response is polyclonal there is possibility of spill over so that some unaltered epitopes adjacent to the original carbonyl modification epitopes which are not normally recognized, now become so-thereby breaking the tolerance to these unaltered epitopes. In this way, B-cells may start producing altered antibodies in response to cigarette smoke which occurs in patients with COPD (Lee *et al.*, 2007; Feghali-Bostwick *et al.*, 2008). However, the pathways responsible for abnormal antibody production from B-cells in response to cigarette smoke are not known. We have recently showed that decreased RelB in B-cells in response to cigarette smoke would be the key in signaling for altered antibody production (Yang *et al.*, 2009).

Cytokines and chemokines

Chronic inflammation is one of the hallmarks of COPD. Lung inflammatory and structure cells are known to release or produce proinflammatory cytokines including TNF- α , interleukins (IL)-1 β , 6, and 8 and interferons (IFNs) (De Boer, 2002). Previous reports describe elevated levels of IL-1 β , IL-6, CXCL8/IL-8, GM-CSF and TNF- α in either induced sputum or BAL fluid or released from alveolar macrophages exposed to cigarette smoke in patients with COPD (Keatings *et al.*, 1996; Pesci *et al.*, 1998; Vlahos *et al.*, 2006; Saha *et al.*, 2009). Sputum neutrophils counts and level of IL-8 and circulating level of TNF- α are the best markers relating to the severity of COPD (Franciosi *et al.*, 2006). However, the antibodies against IL-8 and TNF- α had little clinical effect in patients with COPD (Mahler *et al.*, 2004; Rennard *et al.*, 2007; Dentener *et al.*, 2008). On the contrary, the increased risk of malignancies was seen in TNF- α antibody (infliximab)-treated subjects (Rennard *et al.*, 2007). Hence, it is crucial to develop the cytokines/chemokines antagonists/antibodies with high selectivity and low toxicity for the intervention of COPD.

Recent studies highlighted the importance of IL-18 in pathogenesis of COPD. Cigarette smoke caused activation of IL-18 signaling pathway in mice and human, and IL-18 protein was strongly expressed in alveolar macrophages, CD8⁺ T-cells, and both the bronchiolar and alveolar epithelial cells in the lungs of COPD patients (Kang *et al.*, 2007; Petersen *et al.*, 2007; Imaoka *et al.*, 2008). Furthermore, serum level of IL-18 in patients with GOLD stage III and IV COPD was significantly higher than in smokers and nonsmokers. There was a significant negative correlation between serum IL-18 level and the predicted FEV₁ in patients with COPD. Importantly, knockout of IL-18 receptor α subunit attenuated cigarette smoke or cigarette smoke/poly (I:C)-mediated emphysema and lung inflammatory response in mice (Kang *et al.*, 2007; Kang *et al.*, 2008) whereas constitutive overproduction of IL-18 in the lungs induced the emphysema and lung inflammatory response in mice suggesting the pro-inflammatory and pro-emphysematous effect of IL-18 (Hoshino *et al.*, 2007). It has been shown that IL-18 plays an important role in Th1 polarization and various Th1-type diseases (Dinarello, 1999; Gracie *et al.*, 2003). Indeed, IL-18 also potentially induced Th2 cytokines (IL-4, IL-5, IL-10, and IL-13), IgE, and IgG1 production (Hoshino *et al.*, 1999; Hoshino *et al.*, 2000; Hoshino *et al.*, 2007). Furthermore, targeting of IL-13 to the adult lung caused emphysema in mice via a MMP- and cathepsin-dependent mechanism indicating Th2 inflammatory response also participates in the pathogenesis of COPD (Zheng *et al.*, 2000; van der Pouw Kraan *et al.*, 2002; Miotto *et al.*, 2003). Cigarette smoke selectively enhances viral pathogen-associated molecular pattern (PAMP)-induced pulmonary inflammation, apoptosis and remodeling leading to emphysema in mice (Kang *et al.*, 2008). This effect was mediated by early induction of type I IFN and IL-18, and later induction of IL-12/IL-23 p40 and IFN- γ , and the activation of double-stranded RNA-dependent protein kinase (PKR) and

eukaryotic initiating factor-2 α . This study suggests that cigarette smoke selectively augments the airway and alveolar inflammatory and remodeling responses induced by viral PAMPs and viruses in mouse lung.

IL-32 is a pro-inflammatory cytokine produced by T lymphocytes, natural killer cells, epithelial cells, and blood monocytes (Kim *et al.*, 2005; Netea *et al.*, 2005). It induces other pro-inflammatory cytokines/chemokines, such as TNF- α , IL-1 β , IL-6, and IL-8 by means of the activation of NF- κ B and p38 MAPK (Kim *et al.*, 2005; Netea *et al.*, 2005). Previous study showed that the level/expression of IL-32 was increased in lung tissue of patients with COPD, where it was co-localized with TNF- α and correlated with the degree of airflow obstruction (Calabrese *et al.*, 2008). These results suggest that IL-32 is indeed implicated in the characteristic immune response of COPD, with a possible impact on disease progression.

Chemokines can be subdivided into four subfamilies based on their structural homology around 4 cysteine residues: -C-, -CC-, -CXC-, and -CX3C-, in which X substitutes for any amino acid. They act via specific membrane-bound receptors resulting in the activation of signal transduction pathways that lead to chemotaxis or other activities including proliferation, differentiation, and survival. There are several chemokines including -CXC- (CXCL1, CXCL5, CXCL7-11), and -CC- (CCL2-5, CCL7, CCL8, CCL11, CCL13) involved in the recruitment of inflammatory cells in COPD (Lukacs *et al.*, 2005; Donnelly and Barnes, 2006) (Fig. 2). Therefore, inhibition of chemokine signaling such as chemokine-receptor antagonists would be a potential approach for COPD therapy.

CXCL1 (GRO α) and CXCL8/IL-8 are produced by both structural and inflammatory cells including macrophages. Both of these chemokines bind to their receptor CXCR2 whereas CXCL8/IL-8 binds also to CXCR1. CXCL5 (epithelial cell-derived neutrophil-activating peptide-78) is derived predominantly from epithelial cells and also activates CXCR2. CXCR1 and CXCR2 are expressed on neutrophils while CXCR2 is also expressed on other inflammatory cells including a subset of CD8⁺ T cells, mast cells and macrophages. CXCL1, CXCR5, and CXCL8 are chemotactic and activate inflammatory cells while CXCL8/IL-8 induces neutrophils to degranulate, and causes an oxidative burst. The levels of CXCR1, CXCR5, and CXCR8 were significantly increased in induced sputum and BAL fluid of patients with COPD compared with normal smokers and non-smokers (Keatings *et al.*, 1996; Morrison *et al.*, 1998; Soler *et al.*, 1999; Traves *et al.*, 2002). Neutralization of CXCL8 with a blocking antibody significantly reduced the neutrophil chemotactic activity of sputum from patients with COPD (Beeh *et al.*, 2003). However, this antibody had little clinical effect in patients with COPD (Mahler *et al.*, 2004) promoting the study of small molecular inhibitors of CXCR2 for therapy against the progression of COPD (Widdowson *et al.*, 2004). In mice exposed to acute cigarette smoke, a CXCR2 antagonist (SCH-N) decreased the neutrophilic inflammatory response in lungs, however the compound itself caused neutropenia (Thatcher *et al.*, 2005). At present, CXCR2 antagonists are undergoing Phase I and II trials, the efficacy of these treatments can be assessed once these trials have been reported.

The levels of CXCR3 chemokines CXCL9 (monokine induced by IFN- γ), CXCL10 (IFN- γ inducible protein 10, IP-10), and CXCL11 (IFN-inducible T-cell α chemoattractant) were significantly increased in the sputum of patients with COPD when compared with non-smokers but not with chronic smokers without airway obstruction (Costa *et al.*, 2008) (Fig. 2). Furthermore, increased expression of these chemokines receptor (CXCR3) and its ligand (CXCL10) have been shown in the lungs of COPD patients (Saetta *et al.*, 2002; Hardaker *et al.*, 2004). Interestingly, most CXCR3 positive cells coexpressed CD8⁺ T cells in lungs of COPD patients (Saetta *et al.*, 2002). Moreover, a basal-to-apical gradient of CXCL11 across the epithelium was markedly increased in lungs of patients with COPD, and this increase

may stimulate transepithelial migration of T lymphocytes across the intact bronchial epithelial monolayers suggesting that an increase in CXCR3 chemokines may lead to T lymphocytes accumulation in COPD (Porter *et al.*, 2008). CXCR3 are also expressed on B-lymphocytes and hence may account for increased B-cell follicles that are associated with more severe COPD (Hogg *et al.*, 2004b).

CCL2 (monocyte chemotactic protein-1, MCP-1) is produced by a variety of cells including macrophages, T cells and epithelial cells, and can activate CCR2 on monocyte and T cells. The level of CCL2 was increased in the sputum, BAL fluid and lung of patients with COPD (Capelli *et al.*, 1999; de Boer *et al.*, 2000; Traves *et al.*, 2002) suggesting an important role of CCL2 in the pathogenesis of COPD. CCL2 is also involved in tissue remodeling. CCL2 and its receptor (CCR2) are shown to be directly involved in endothelial and lung epithelial cell proliferation, migration and wound closure *in vitro* (De Boer *et al.*, 2007a; De Boer *et al.*, 2007b). In addition, CCL2 stimulates collagen synthesis in rat lung fibroblasts via a TGF β 1-dependent pathway and hence potentially contribute to a fibrogenic remodeling as seen in COPD. Recent studies demonstrated that CCR5 level was increased in lungs of COPD patients, and silencing of CCR5 attenuated cigarette smoke-induced lung inflammation and emphysema in mouse (Bracke *et al.*, 2007; Costa *et al.*, 2008). It is important to note that the enhanced immune response occurred in CCR5-deficient mice which may be due to the higher production of the CCR5 ligands, the overexpression of other pro-inflammatory cytokines (e.g., IL-6, IL-8) and the involvement of CCR5 in T cell apoptosis (Algood and Flynn, 2004; Mojtahedi, 2006; Murooka *et al.*, 2006). However, it remains to be seen whether CCR5 plays the same role in other animal models of COPD/emphysema, and CCR5 (or CCR2/CCR5) antagonist has any beneficial effects in COPD.

CX3CL1 (fractalkine or mouse neurotactin) is the unique member of the CX3C chemokine subfamily. In contrast to other chemokines, it exists in two forms, each mediating distinct biological actions. The membrane-anchored protein, which is expressed primarily on the inflamed endothelium, epithelial cells, dendritic cells, and neurons, serves as an adhesion protein promoting the retention of monocytes and T cells. The soluble form resembles more a conventional chemokine and strongly induces chemotaxis. Chemotaxis and adhesion are mediated by the G protein-coupled receptor CX3CR1 that is expressed by cytotoxic effector CD8⁺ and CD4⁺ T lymphocytes in addition to $\gamma\delta$ T lymphocytes, NK cells, dendritic cells, and monocytes (Combadiere *et al.*, 1998a; Combadiere *et al.*, 1998b; Niess *et al.*, 2005). Recent studies showed that gene expression of CX3CL1 was increased in lungs of smokers with COPD, and mouse lungs in response to chronic cigarette smoke exposure (Ning *et al.*, 2004; McComb *et al.*, 2008). This was associated with recruitment and accumulation of CX3CR1⁺ T lymphocytes and macrophages in the lungs. Therefore, one function of the CX3CR1-CX3CL1 pathway is to recruit and sustain divergent immune cell populations implicated in the pathogenesis of cigarette smoke-induced emphysema.

NF- κ B pathways in cigarette smoke-mediated lung inflammatory response

As mentioned in preceeding paragraphs, the levels of proinflammatory cytokines and chemokines are increased both locally in the lung and systemically in plasma in patients with COPD. Although many transcription factors are involved in the regulation of these inflammatory proteins, NF- κ B is of particular importance (Christman *et al.*, 2000). The genes for these pro-inflammatory mediators which have been involved in inflammatory process of the airways in COPD, including IL-1, IL-6, IL-8, MCP-1, and TNF- α which are all regulated by NF- κ B. Indeed, the numbers of RelA/p65-positive epithelial cells and macrophages and RelA/p65 nuclear expression were increased in smokers and patients with COPD (Di Stefano *et al.*, 2002; Yagi *et al.*, 2006; Rajendrasozhan *et al.*, 2008b). Furthermore, in COPD patients the number of RelA/p65-positive epithelial cells and macrophages correlated with the degree of airflow limitation (Di Stefano *et al.*, 2002). This

suggests that NF- κ B activation plays an important role in chronic inflammatory response seen in COPD. The activation of NF- κ B in lungs of patients with COPD is associated with increased oxidative stress, due to the fact that NF- κ B activating upstream kinases are redox-sensitive (Bowie and O'Neill, 2000; Pantano *et al.*, 2006).

The activation of NF- κ B transcription factors occurs through two main pathways: the canonical and the alternative pathways (Fig. 3). During canonical signaling upstream mediators activate the I κ B kinase (IKK) complex, composed of the two catalytic subunits IKK- α and IKK- β and a third structural subunit, IKK- γ , to phosphorylate inhibitory I κ B proteins, leading to their ubiquitination and degradation. This leads to the disassociation of RelA/p65 with I κ B and translocation of RelA/p65 into nucleus. RelA/p65 can be phosphorylated by a number of kinases such as protein kinase A (PKA) and mitogen- and stress-activated protein kinase (MSK)1 (both at serine 276), IKK- β (at serine 536) and protein kinase C (PKC) ζ (serine 311), each of which leads to an increase in RelA/p65's ability to induce cytokine release (Yang *et al.*, 2003; Chen and Greene, 2004). This is because phosphorylation of the RelA/p65 subunit facilitates binding of CBP/p300, which is able to acetylate RelA/p65 at lysines 218, 221, and 310. In particular, acetylation of lysine 310 is important in pro-inflammatory gene transcription. Previously, we have shown that cigarette smoke exposure induced the inflammatory cells influx which was associated with increased levels of various NF- κ B-dependent pro-inflammatory mediators in lungs, and IKK- β inhibitors attenuated cigarette smoke extract-induced NF- κ B-dependent pro-inflammatory mediators release from peritoneal macrophages and monocyte-macrophage MonoMac6 cells (Yang *et al.*, 2006; Yao *et al.*, 2008b). The alternative or noncanonical pathway requires the NF- κ B-inducing kinase (NIK), which cooperates with IKK- α to induce the processing of the p100 C-terminus (termed I κ B δ), which results in the nuclear translocation of p52:RelB (Senftleben *et al.*, 2001; Yin *et al.*, 2001; Xiao *et al.*, 2004). Cigarette smoke exposure increased the levels of p52, RelB, IKK- α and NIK as well as RelB interaction of p52 with NIK in mouse lung (Yang *et al.*, 2008; Yang *et al.*, 2009). This was associated with recruitment of RelB on the promoter of pro-inflammatory genes suggesting that alternative NF- κ B pathway also participates in cigarette smoke-mediated lung inflammatory response (Yang *et al.*, 2008). Furthermore, cigarette smoke-mediated NF- κ B activation (increased phosphorylated ser276 and acetylated lys310 on RelA/p65, nuclear level of RelB, and IL-8 release) was augmented in MonoMac6 cells transfected with IKK- α (Yang *et al.*, 2008) implicating IKK- α is required for NF- κ B activation by cigarette smoke. This was consistent with previous studies which showed that the pro-inflammatory cytokine-induced NF- κ B-dependent transcription, and promoter activation was markedly decreased in IKK- α deficient fibroblasts even though I κ B α degradation and NF- κ B *in vitro* DNA binding activity were normal in these cells in response to TNF- α or IL-1 (Sizemore *et al.*, 2002; Anest *et al.*, 2003; Yamamoto *et al.*, 2003). Further study showed that cigarette smoke-mediated activation of IKK- α caused histone modification (phosphorylation and acetylation of histone 3) and recruited acetylated RelA/p65 and other co-activators such as CBP on promoters of proinflammatory genes leading to sustained proinflammatory mediators release (Yang *et al.*, 2008). As another component of NF- κ B pathway, RelB was also recruited on proinflammatory genes promoters via NIK and/or IKK- α activation by cigarette smoke in MonoMac6 cells. However, it is interesting to note that RelB was degraded rapidly by proteolysis in B lymphocytes in response to cigarette smoke suggesting RelB is differentially regulated by cigarette smoke in cell specific manner and speculating the pro- and anti-inflammatory protective role of RelB which is cell type-specific (Yang *et al.*, 2009). Furthermore, RelB degradation in B cells may signal for RelA/p65 activation leading to proinflammatory cytokines release, and/or alter the acquired immunity resulting in abnormal/self antibody production.

In addition to IKK, NF- κ B activation is also regulated by MSK1 by phosphorylating RelA/p65 (serine 276) and histone H3, to establish a transcription-competent promoter complex (enhanceosome) (Vermeulen *et al.*, 2003). Cigarette smoke extract increased the level of MSK1 in MonoMac6 cells but its level was decreased in IKK- α -knockdown MonoMac6 cells (Yang *et al.*, 2008) suggesting MSK1 may play an important role in IKK- α -mediated NF- κ B activation and chromatin modifications on proinflammatory gene promoters in response to cigarette smoke (Fig. 2). However, further studies are required to confirm this contention using knockdown or overexpression models of MSK1 *in vitro* and *in vivo*.

The PKC family of serine/threonine kinases is ubiquitously expressed and is divided into three categories based on the cofactors required for their activation. The activation of conventional PKC members is dependent on calcium and diacylglycerol, novel members are calcium independent but activated by diacylglycerol, and the atypical family members do not require calcium or diacylglycerol. Recent studies show that PKC ζ , an atypical family member of PKC, regulates the activation of NF- κ B via activating IKK, stabilizing I κ B- α , and/or directly phosphorylating RelA/p65 (ser311) in lungs of mice intraperitoneally injected with TNF- α , LPS or IL-1 (Leitges *et al.*, 2001; Duran *et al.*, 2003) (Fig. 3). Importantly, activated PKC ζ also participates in cigarette smoke extract-mediated apoptosis in human fetal lung fibroblast (MRC-5) cells, and its expression is elevated in lungs of patients with COPD (Park *et al.*, 2008) suggesting PKC ζ /IKK/NF- κ B pathway plays an important role in pathogenesis of COPD. Indeed, our preliminary data showing decreased lung inflammatory response in PKC ζ -knockout mice exposed to cigarette smoke or LPS (Yao *et al.*, unpublished data). These results suggested that PKC ζ is an important modifier of lung inflammatory response, and down-modulation of PKC ζ may have novel therapeutic potential in prevention of cigarette smoke-related lung diseases.

EPIGENETICS IN PATHOGENESIS OF COPD

Epigenetics is the term used to describe heritable changes in gene expression that is not coded in the DNA sequence itself but it is governed by post-translational modifications in histone proteins and DNA. These modifications include chromatin remodeling (histone acetylation, methylation, ubiquitination, phosphorylation, and sumoylation) and DNA methylation.

Histone acetyltransferase and deacetylase in lung inflammation

The complex structure of chromatin consists of DNA wrapped around an octamer of core histones, which is composed of two molecules of each of the histones H2A, H2B, H3, and H4. Acetylation of lysine residues on the N-terminal tails of the core histone proteins results in uncoiling of the DNA, allowing increased accessibility for transcription factor binding leading to gene transcription (Imhof and Wolffe, 1998; Rahman *et al.*, 2004; Ito *et al.*, 2007). It is known that acetylation of core nucleosomal histones is regulated by the opposing activities of HATs and HDACs.

HATs in lung inflammation

HATs are divided into five families. These include the Gcn5-related acetyltransferase (GNATs), the MYST-related HATs, p300/CBP HATs, the general transcription factor HATs, which include the TFIID subunit TAF250, the nuclear hormone-related HATs SRC1 and ACTR (Carrozza *et al.*, 2003). Of these, p300/CBP which is regulated by the p38 MAP kinase pathway, is vital for the co-activation of several transcription factors including NF- κ B and AP-1 in the transcription machinery (Thomson *et al.*, 1999). Thus, it is likely that histone acetylation via CBP/p300 has a significant role in the activation of NF- κ B/AP-1-mediated gene expression for pro-inflammatory mediators (Kamei *et al.*, 1996; Carrero *et*

et al., 2000). Interestingly, NF- κ B also induces histone acetylation in a temporal manner leading to the recruitment of other co-activators and remodeling complexes and the induction of proinflammatory gene expression although it is, itself, acetylated by other HATs (Ito *et al.*, 2000; Ghosh and Karin, 2002; Lee *et al.*, 2006). NF- κ B-induced acetylation occurs preferentially on histone H4, rather than histones H2A, H2B or H3, in epithelial cells and is directed primarily towards lysine 8 and 12 at NF- κ B responsive regulatory elements on proinflammatory genes (Ito *et al.*, 2000).

HDAC2 in lung inflammation

The family of HDAC enzymes consists of 17 isoforms grouped into four families (de Ruijter *et al.*, 2003). Class I HDACs (HDAC1-3 and 8) reside almost exclusively in the nucleus, whereas class II HDACs (HDAC4-7, 9-10) are able to shuttle between the nucleus and cytoplasm in response to certain cellular signals. The third HDAC family consists of sirtuins 1-6 and their function is not yet fully understood. HDAC11 belongs to class IV. A common feature of HDACs is the ability to remove acetyl moieties from the ϵ -acetamido group on lysine residues within histones, resulting in condensation of DNA thereby silencing gene transcription. HDACs not only deacetylate histones but also have the ability to deacetylate non-histone proteins such as NF- κ B and thereby have the ability to regulate NF- κ B-dependent pro-inflammatory gene transcription (Sengupta and Seto, 2004). We have shown that cigarette smoke-mediated reduction in HDAC2 was associated with increased levels of total and acetylated RelA/p65, and indicated RelA/p65 interacts with HDAC2 and RelA/p65 becomes available or retained in the nucleus for pro-inflammatory gene transcription when HDAC2 is decreased (Yang *et al.*, 2006; Yao *et al.*, 2008a). Furthermore, HDAC inhibitor trichostatin A has been reported to enhance NF- κ B-driven inflammatory gene transcription in cell lines (Ito *et al.*, 2000; Chen *et al.*, 2001). Therefore, alteration of HDACs by cigarette smoke leads to acetylation of histones and transcription factor such as NF- κ B, resulting in the increased transcription of proinflammatory genes (Yang *et al.*, 2006; Adenuga *et al.*, 2008a; Adenuga *et al.*, 2008b).

Importantly, there is a marked reduction of HDAC2 expression/activity in lung parenchyma, bronchial biopsies and alveolar macrophages of patients with COPD, and this decrease is correlated with disease severity and the intensity of inflammation (Ito *et al.*, 2005). The mechanism underlying the reduction of HDAC2 level/activity is associated with its posttranslational modifications such as nitrosylation, phosphorylation and ubiquitination leading to proteasome-dependent degradation particularly in response to cigarette smoke (Galasinski *et al.*, 2002; Adenuga *et al.*, 2009), or due to oxidative/carbonyl modifications of HDAC2 (Yang *et al.*, 2006). HDAC2 is required for the anti-inflammatory effects of glucocorticoids as reduced levels/activity of HDAC2 has been shown to occur in patients with COPD with subsequent corticosteroid resistance (Ito *et al.*, 2005). Elevation of HDAC activity by curcumin and theophylline significantly enhanced steroids suppression of induced IL-8 release in monocytes and alveolar macrophage from patients with COPD which was blocked by the HDAC inhibitor trichostatin A (Cosio *et al.*, 2004; Meja *et al.*, 2008). Furthermore, HDAC2 can deacetylate glucocorticoid receptor (GR), thereby enabling the association of GR with RelA/p65, and subsequently attenuate pro-inflammatory gene transcription (Ito *et al.*, 2006). Therefore, restoration or attenuation of HDAC2 loss will enhance glucocorticoid sensitivity by deacetylating the RelA/p65 and GR. Such restoration is possible by reversing the posttranslational modifications of HDAC2 such as decarbonylation or dephosphorylation via inducing aldehyde dehydrogenases/reductases and phosphatases, or inducing the antioxidant buffer systems using Nrf2 activators and ECSOD mimetics.

Sirtuin 1 (SIRT1) in lung inflammation

Sirtuin 1 (SIRT1) is a class III HDAC with anti-inflammatory, anti-aging/senescence, and anti-apoptotic activity mediated by the deacetylation of histones and non-histone proteins including transcription factor (FOXO, p53, and NF- κ B) (Yang and Sauve, 2006). We have shown that the level of SIRT1 is reduced in rat lungs and MonoMac6 cells (Yang *et al.*, 2007) and in lungs of human smokers and patients with COPD (Rajendrasozhan *et al.*, 2008b) implicating the pivotal role of SIRT1 in the pathogenesis of COPD. The reason for SIRT1 reduction is due to its posttranslational modifications such as carbonylation and phosphorylation leading to degradation in response to cigarette smoke/oxidative/carbonyl stress (Caito *et al.*, 2008; Rajendrasozhan *et al.*, 2008b). Knockdown of SIRT1 with siRNA leads to an increased activation of NF- κ B and subsequent inflammatory response whereas upregulation of SIRT1 by SRT1720 and resveratrol inhibited pro-inflammatory mediators release in response to cigarette smoke exposure (Rajendrasozhan *et al.*, 2008b) suggesting modulation of SIRT1 with activators or endogenous regulators (Milne *et al.*, 2007; Milne and Denu, 2008) would be an approach for the intervention of COPD. This contention is conformed by the study showing the attenuation of cigarette smoke-induced lung inflammation in mice after SIRT1 activator (i.e. SRT2172) administration (Nakamaru *et al.*, 2009). The protection against lung inflammatory and injurious responses by SIRT1 is associated with the deacetylation of RelA/p65 and negative regulation of MMP-9 in response to cigarette smoke/oxidative stress (Chen *et al.*, 2002; Yang *et al.*, 2007; Nakamaru *et al.*, 2009). However, further study is required to investigate whether SIRT1 regulates the progression of COPD/emphysema using genetic/pharmacological approaches.

SIRT1 also deacetylates other transcription factors such as forkhead box class (FOXO3) and p53 thereby regulating oxidative stress-induced cell cycle arrest, apoptosis and cellular senescence which play an important role in the pathogenesis of COPD. We have shown that FOXO3 is acetylated when SIRT1 is reduced in response to cigarette smoke exposure in mouse lung (Rajendrasozhan *et al.*, 2008a). Therefore, the study on SIRT1-FOXO3 pathway will further elucidate the pathological mechanisms, and provide the possible therapeutic targets for COPD. SIRT1 interacts with p53 and deacetylates its C-terminal regulatory domain (Vaziri *et al.*, 2001), whereas reduction of SIRT1 leads to increased acetylation of p53 thereby increasing its pro-apoptotic function and cellular senescence (Vaziri *et al.*, 2001; Luo *et al.*, 2004). Oxidative stress accelerates cellular senescence by accumulation of acetylated p53 via decrease in the function of SIRT1 by NAD⁺ depletion (Furukawa *et al.*, 2007; Ota *et al.*, 2007). Moreover, blockade of p53 by antisense oligonucleotides reversed the inhibitory effect of SIRT1 on cellular senescence (Ota *et al.*, 2007). Our previous study showed that nuclear SIRT1 levels were decreased *in vivo* and *in vitro* in response to cigarette smoke exposure (Yang *et al.*, 2007), but it is not known if SIRT1-mediated regulation of p53 (acetylation) plays a role in cigarette smoke-mediated apoptosis and senescence. Similarly, SIRT6 is also implicated in inflammatory response, senescence and aging (Michishita *et al.*, 2008; Kawahara *et al.*, 2009; Van Gool *et al.*, 2009) and hence other SIRT members gain equal credence in understanding the pathogenesis of COPD.

Endothelial cells dysfunction plays a pivotal role in pathogenesis of emphysema, and cigarette smoke-induced emphysematous alveolar septa are almost avascular which is associated with reduced expression of endothelial nitric oxide synthase (eNOS) and endothelium dysfunction (Yamato *et al.*, 1996; Kasahara *et al.*, 2001; Edirisinghe *et al.*, 2008; Wright and Churg, 2008; Ferrer *et al.*, 2009). Recent studies showed that SIRT1 is a key regulator of vascular endothelial homeostasis controlling angiogenesis, vascular tone and endothelial dysfunction by regulating eNOS (Potente and Dimmeler, 2008a). Furthermore, SIRT1 has been shown to bind to eNOS, and deacetylate lysines 496 and 506 in the calmodulin-binding domain of eNOS leading to enhanced nitric oxide (NO) production which is an essential for endothelial-dependent vasorelaxation, endothelial cell survival,

migration and postnatal neovascularization (Mattagajasingh *et al.*, 2007). It is interesting to note that NO has been shown to activate the SIRT1 promoter leading to an increase of SIRT1 mRNA and protein (Nisoli *et al.*, 2005; Ota *et al.*, 2008) indicating that a positive feedback mechanism exists between SIRT1 and eNOS (Potente and Dimmeler, 2008b). Furthermore, Thus, activating SIRT1 through small molecules may help to reset the activity of eNOS during situations of endothelial dysfunction where NO availability is limited in smokers (Michaud *et al.*, 2006). Moreover, cigarette smoke-induced apoptosis of coronary arterial endothelial cells and inflammatory response were attenuated by SIRT1 overexpression (Csiszar *et al.*, 2008). Therefore, SIRT1 is a possible molecular target to prevent and/or treat pulmonary and cardiovascular diseases including COPD (emphysema) and atherosclerosis by protecting endothelial cells from stress-induced premature senescence, apoptosis and inflammatory response.

Histone/DNA methylation in lung inflammation

Histones can be methylated on either lysine (K) or arginine (R) residues, which is catalyzed by enzymes belonging to three distinct families of protein-the PRMT1 family, the SET-DOMAIN-containing protein family, and the non-SET-domains DOT1/DOT1L (Zhang and Reinberg, 2001; Bannister and Kouzarides, 2005). It is believed that methylation of K or R residues forms a binding site or interacting domain allowing other regulatory proteins to be recruited. Unlike acetylation, which generally correlates with transcriptional activation, histone lysine methylation can signal either activation or repression, depending on the sites of methylation (Zhang and Reinberg, 2001). Furthermore, a cross-talk between different histone modifications also controls gene transcription epigenetically (Cheung and Lau, 2005; Wang *et al.*, 2008). Therefore, positive and negative cross-talks ultimately generate the complex patterns of gene- or locus-specific histone marks which are associated with distinct chromatin states, leading to transcriptional repression or activation.

DNA methylation is another mechanism associated with epigenetic silencing, and this effect is in part mediated by recruitment of HDACs through the methyl-DNA binding motifs of components of several HDAC-containing complexes (Nan *et al.*, 1998). It has been shown that methylation of the promoter regions in multiple genes has been reported in adenocarcinomas and non-small cell lung cancer, and this methylation was associated with tumor progression (Zochbauer-Muller *et al.*, 2001). Therefore, determination of specific gene DNA methylation may provide the useful markers for early detection and/or chemoprotective intervention in cancer. Methylation of p16 promoter was frequent in sputum of patients with COPD, and this methylation was significantly correlated with heavy cigarette smoking suggesting DNA methylation is associated with cigarette smoke-mediated lung diseases (Georgiou *et al.*, 2007). However, little data is available about the histone/DNA methylation in cigarette smoke-induced lung inflammation and emphysema. Further studies on histone/DNA methylation will bring the prospect of new biomarkers and/or treatment for COPD/emphysema.

CONCLUSIONS AND FUTURE DIRECTIONS

Oxidative stress is critical for lung inflammatory response to cigarette smoke/environmental pollutants through the upregulation of redox-sensitive transcription factors, and induction of autophagy and unfolded protein response. Hence, development of antioxidants/thiol agents or other pharmacological agents such as enzyme mimetics-ECSOD or Nrf2 activator or reversing its post-translational modifications by aldehyde dehydrogenases/reductases to boost the endogenous antioxidant system could be used to ameliorate chronic inflammatory and injurious responses in COPD. Further studies on canonical/alternative NF- κ B pathway and their upstream kinases will identify novel therapeutic targets for the intervention of COPD. Since epigenetic modifications (histone acetylation/deacetylation and histone

methylation) are thought to be the mechanism for understanding abnormal inflammation in the pathogenesis and steroid resistance in COPD, it is believed that epigenetic drugs will bring novel avenues for treatment of COPD.

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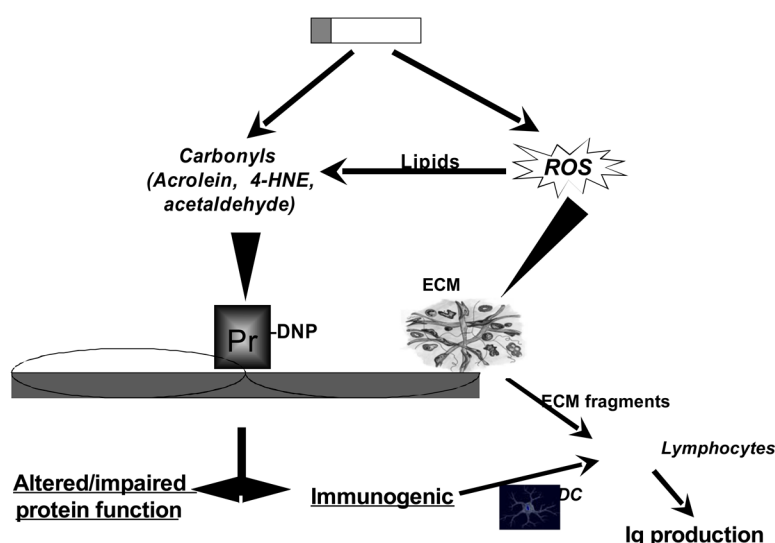


Figure 1. Aldehyde/carbonyl stress in COPD

Cigarette smoke contains different carbonyl compounds which can carbonylate proteins through direct amino acid oxidation or an indirect mechanism involving lipids oxidation leading to formation of reactive aldehydes such as acrolein and 4-hydroxy-2-nonenal (4-HNE). These aldehyde-protein adducts will alter the function and stability of intracellular (e.g. histone deacetylases, nuclear erythroid-related factor 2, or Keap1) or extracellular [e.g. extracellular matrix (ECM)] proteins or cause a variety of cellular and biochemical effects including immunogenicity thereby inducing lung inflammatory and autoimmune responses, and injury. DNP: 2,4-dinitrophenyl, denotes protein carbonylation.; DC: dendritic cells.

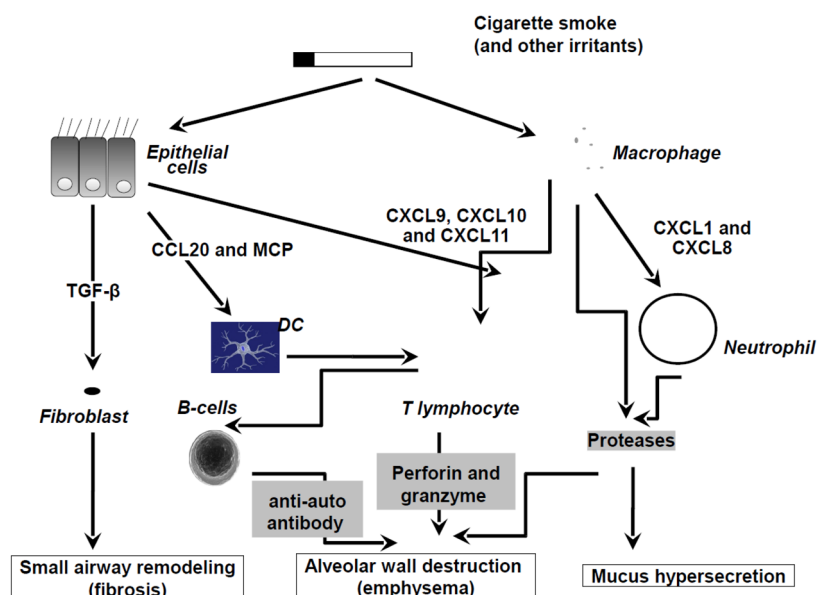


Figure 2. Inflammatory and immune cells involved in COPD

Exposure to cigarette smoke or other irritants activates macrophage and epithelial cells to release chemokines which attract other inflammatory and immune cells including neutrophils, T-cells, dendritic cells (DC), and B-cells into the lungs. CXCL1 and CXCL8 chemokines act on CXCR2 to attract neutrophils while CXCL9, CXCL10 and CXCL11 chemokines bind to CXCR3 to attract T-cells into lungs. Cigarette smoke-induced recruitment of immature DC fails to induce appropriate T-cells response but instead leads to predominantly CD8⁺ T-cells proliferation in lungs. Furthermore, prolonged exposure to cigarette smoke leads to the accumulation of extracellular matrix fragments which are presented by DC to T-cells activating specific B-cells. This will result in the production of auto-antibody, such as anti-elastin body leading to abnormal autoimmunity in lungs. These inflammatory and immune cells release proteases, perforin, granzyme, and produce anti-self antibody causing alveolar wall destruction. Neutrophil-derived elastase also causes mucus hypersecretion. Epithelial cells and macrophages also release transforming growth factor-β (TGF-β) leading to small airway remodeling.

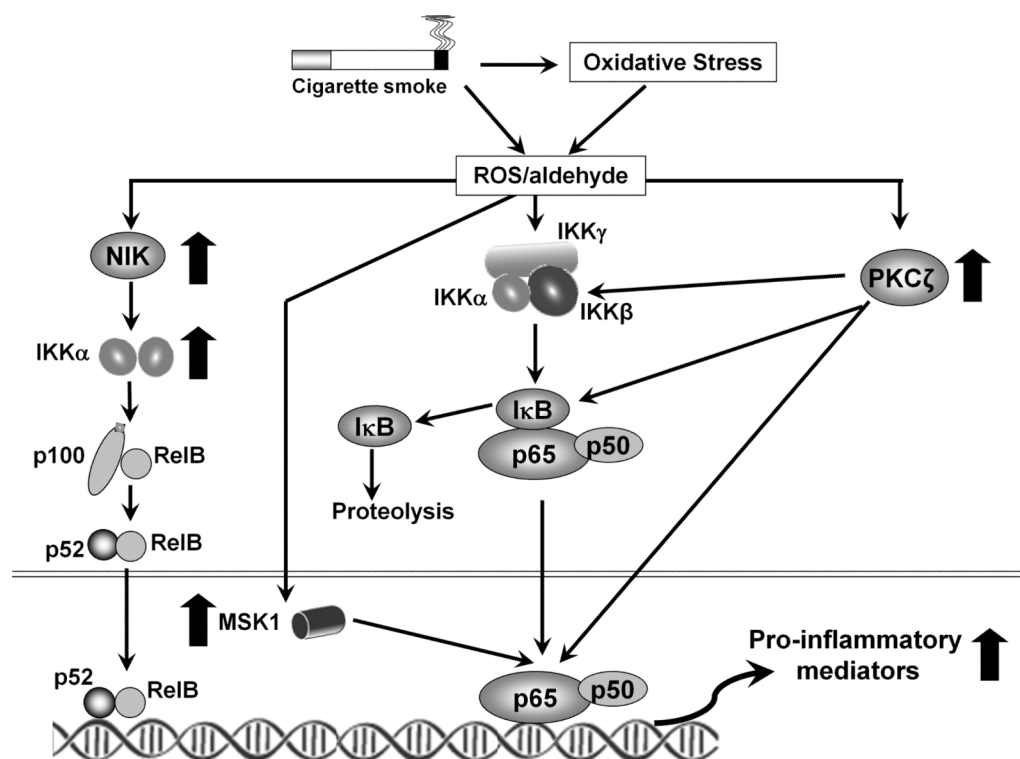


Figure 3. Mechanism of cigarette smoke-mediated activation of NF-κB and pro-inflammatory gene transcription

Cigarette smoke-mediated oxidative stress can activate the IKK complex to phosphorylate inhibitory IκB proteins, resulting in their ubiquitination and degradation. This leads to the translocation of RelA/p65 into nucleus which is recruited on the promoter of pro-inflammatory genes. Alternative NF-κB pathway is also activated in response to cigarette smoke exposure through the cooperation of NIK with IKK-α to induce the processing of the p100 C-terminus resulting in the nuclear translocation of p52:RelB. Furthermore, IKKα-activated MSK1 in response to cigarette smoke induces NF-κB activation by phosphorylating RelA/p65 and altering chromatin modification. PKCζ, an atypical family member of PKC, regulates the activation of NF-κB via activating IKK, stabilizing IκB-α, and/or directly phosphorylating RelA/p65 in response to stimuli. NIK, NF-κB inducing kinase; IKK, IκB kinase; PKCζ, protein kinase Cζ.